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Nail fold capillary diameter changes in acute systemic hypoxia

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ABSTRACT

The present study was undertaken to determine the effect of arterial blood hypoxemia induced by acute systemic hypoxia ($pO_2 = 12\%$) on capillary recruitment and diameter, and red blood cell (RBC) velocity in human nail fold capillaries during rest, arterial post-occlusive reactive hyperemia (PRH), and venous occlusion (VO) using intravital video-capillaroscopy. Capillary recruitment was unchanged in acute systemic hypoxia (H) versus normoxia (N). There was no difference in RBC velocity measurements between normoxia and hypoxia ($P < 0.63$). However, a statistically significant increase in nail fold capillary total width (N, 39.9 ± 9.1 vs. H, 42.7 ± 10.3 μm; $P < 0.05$), apical diameter (N, 15.5 ± 4.3 vs. H, 16.8 ± 4.3 μm; $P < 0.05$), arterial diameter (N, 11.9 ± 3.5 vs. H, 13.9 ± 4.1 μm; $P < 0.05$), and venous diameter (N, 15.5 ± 4.3 vs. H, 17.2 ± 4.8 μm; $P < 0.05$) was observed and continued to be significant most often during post-occlusive reactive hyperemia (PRH) and venous congestion (VO). These data suggest that acute systemic hypoxia does not increase capillary recruitment, but instead increases capillary diameter, resulting in increased capillary blood flow.

Introduction

Blood flow in the microcirculation plays an important role in maintaining healthy tissues and organs by delivering oxygen and nutrients (Jeong et al., 2006). The influence of hypoxia on skin blood flow is poorly understood. It has been suggested that this stimulus causes vasodilation in human skin (Simmons et al., 2007), but the mechanisms of this phenomenon are at the microcirculatory level, such that moderate decreases in oxygen delivery cause dilation of the terminal arterioles, thereby allowing a more homogeneous distribution of oxygen through the capillary network and the diffusion of oxygen to the target tissue (Marshall and Davies, 1999). In contrast, it is known that acute exposure to hypoxia evokes changes in local vasodilator and neural vasoconstrictor factors that significantly influences vascular tone. In healthy human studies (Dinenno et al., 2003), mild-to-moderate systemic hypoxia doesn't blunt the sympathetic vasoconstriction via α-adrenergic receptors. However, recent evidence suggests that the responsiveness of the sympathetic adrenergic system can be modulated by factors associated with the cutaneous active vasodilator system (Shibasaki et al., 2008). During systemic hypoxia, when sympathoadrenal influence on vascular tone is eliminated, blood flow in the forearm is controlled by local vasodilator mechanisms (Markwald et al., 2011). The same effect is observed in cutaneous vasculature (Simmons et al., 2007). Moreover, when oxygen delivery falls below a critical value, oxygen utilization becomes delivery dependent and decreases in a linear fashion (Curtis et al., 1995). During normoxia, oxygen is supplied to the tissue mostly by arterioles, whereas in hypoxia, oxygen is supplied to tissues by capillaries through a NO concentration-dependent mechanism that controls capillary perfusion and tissue pO_2 (Bertuglia and Giusti, 2005). Therefore, this study was performed in order to determine the effect of arterial blood hypoxemia induced by acute systemic hypoxia ($pO_2 = 12\%$) on capillary recruitment, capillary diameter, and red blood cell (RBC) velocity in human nail fold capillaries during rest, arterial post-occlusive reactive hyperemia (PRH), and venous occlusion (VO).

Material and methods

Subjects

The study included nineteen healthy young adult (8 women and 11 men) volunteers (Table 1) who did not have a history of peripheral vascular pathology such as Raynaud's syndrome, dermatologic diseases, or systemic diseases such as diabetes or hypertension. All respondents were non-smokers. The subjects were familiarized with the experimental procedures and provided written informed consent according to the Declaration of Helsinki. The study protocol was approved by the Scientific Investigation Ethics Commission of the University of Latvia Institute of Experimental and Clinical Medicine.

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Table 1
Characteristics of study subjects.

Characteristics		
Subjects		
Total, n		19
Male, n		11
Female, n		8
Age, y		21 ± 2.0
Body mass index, kg/m^2		25 ± 3.6
Characteristics	Normoxic conations (pO_2 = 21%)	Hypoxic conditions (pO_2 = 12%)
SBP, mmHg	123 ± 11.7	127 ± 12.3*
DBP, mmHg	70 ± 10.0	71 ± 11.4
MAP, mmHg	88 ± 9.6	89 ± 10.1
HR, bpm	65 ± 15.3	73 ± 16.5†
SpO_2, %	97 ± 1.3	78 ± 4.6†
$TcpO_2$, mmHg	60 ± 15.6	14 ± 9.6†
$TcpCO_2$, mmHg	69 ± 8.2	71 ± 14.8

Values are mean ± SD. SBP indicates systolic blood pressure; DBP, diastolic blood pressure, MAP, mean arterial pressure; HR, heart rate; SpO_2, Oxygen saturation; $TcpO_2$, Transcutaneous oxygen tension; and $TcpCO_2$, Transcutaneous carbon dioxide tension. *P < 0.05, †P < 0.001 from normoxic vs. hypoxic conditions used Wilcoxon ranked-sum test.

Experimental condition and protocol

To simulate systemic hypoxia (normobaric hypoxia), a hypoxicator (GO2Altidude, Biomedtech, Melbourne, Australia) which has an air separation system employing semi-permeable membrane technology (Spurling et al., 2011) was used, continuously pumping air at a flow rate of 20 l/min^{-1} into an air bag which was connected to a facial mask to deliver lower atmospheric O_2 concentration to the subjects (GO2Altitude, Biomedtech, Melbourne, Australia). Gas concentrations in the bag (oxygen mixture at 12%) were monitored by an oxygen sensor (Cambridge Sensotec, Cambs, UK). Arterial blood oxygenation (SpO_2) and heart rate (HR) were recorded online with a pulse oximeter (GO2Altitude, Biomedtech, Melbourne, Australia). In addition, blood pressure (HEM-711 AC, OMRON Healthcare, Kyoto, Japan) was recorded at every capillary measurement occasion. Gas partial pressure in the skin was continuously recorded by transcutaneous monitors (TCM4, Radiometer, Copenhagen, Denmark). The transcutaneous probe was applied on the same hand 2–3 cm proximal from the thumb.

Intravital video capillaroscopy

Intravital-capillaroscopy was used to visualize surface microvessels in the nail fold area of the right middle finger. Three types of physiological conditions were observed in normoxic and acute systemic hypoxic conditions. First, basal functional capillary density was observed in normoxia and hypoxia after 20 min of acclimatization in a supine position. At this time, collection of resting data was begun. After recording the resting data, arterial occlusion was applied for 3 min, and upon release of the forearm cuff (Hokanson Inc., Bellevue, WA, USA), the response of post-occlusion reactive hyperemia (PRH) was recorded for 30 s (CAM1 L300, CapiScope, KK-Technology, Bridleways Holyford, Devon, England). The reactivity of capillaries was observed after arterial occlusion, and the structural density of capillaries was observed during 2 min of venous congestion (Serne et al., 2001; Penna et al., 2008). Capillary density was defined as the number of erythrocyte-perfused capillaries per square millimeter of nail fold skin (Serne et al., 2001). In all phases the images were stored on video. The number of capillaries was counted offline by two experienced investigators (A.P. and K.N.M) from a freeze-framed reproduction of the video and analyzed using CapiScope Image Acquisition and Analysis software (CapiScope V.3.6.4.0, KK-Technology, Bridleways Holyford, Devon, England). Capillary diameters were measured according to previously described methods by Allen et al. (2003). The percentage change in capillary recruitment was calculated by dividing the absolute change in capillary density during post-reactive hyperemia (PRH) and venous occlusion (VO) by basal capillary density (×100) (Tibirica et al., 2007).

Statistical analysis

The Kolmogorov–Smirnov test was used to establish normality of the data. The differences between normoxia and hypoxia were analyzed using a paired Student's *t* test for normally distributed data or the Wilcoxon ranked-sum test for nonparametric distribution. Significance was accepted at P < 0.05 and all values are expressed as mean ± SEM (standard error of the mean).

Results

Table 1 shows the characteristics of the study subjects. Table 2 shows, respectively, the capillaroscopy data of the subjects' middle finger nail fold obtained at baseline, during PRH and during VO in normoxic (N) conditions (n = 19), in comparison with hypoxic (H) conditions (n = 19). Mean capillary density of the finger at baseline was not different between N and H (22.6 ± 9.3 and 21.6 ± 8.2 capillaries/mm^2; P = 0.13). The same was observed during PRH (N = 24.2 ± 9.5 and H = 22.5 ± 8.3 capillaries/mm^2; P = 0.08) and VO (N = 23.8 ± 9.1 and H = 22.6 ± 8.8 capillaries/mm^2; P = 0.12). There was a slight, but significant capillary recruitment during PRH in N, and no recruitment after the same maneuver in H. A quantitative estimate regarding the increase in capillary blood flow can be provided considering constant velocity (Table 2) and increased diameter (Table 3). In N a significant difference was observed between the mean capillary density at baseline and during PRH (22.6 ± 9.3 and 24.2 ± 9.5, respectively; P < 0.05). In contrast, no difference was observed between the mean capillary density at baseline and during VO (P > 0.05). RBC velocity was not change (N = 0.61 ± 0.26 [n = 88] and H = 0.63 ± 0.24 [n = 87]; P = 0.63) in acute systemic hypoxia. These data are displayed in Table 2.

Table 3 shows nail fold capillary loop diameters in study subjects. There was a statistically significant increase of nail fold capillary loop total width at baseline between N and H (39.9 ± 9.1 vs. 42.7 ± 10.3 μm; P < 0.001), with the same increases observed in the apex (N = 15.5 ± 4.3 vs. H, 16.8 ± 4.3 μm; P = 0.002), arterial limb (N = 11.9 ± 3.5 vs. H = 13.9 ± 4.1 μm; P < 0.001) and venous limb (N = 15.5 ± 4.3 vs. H = 17.2 ± 4.8 μm; P < 0.001). In PRH, the significant increase in overall nail fold capillary loop size (P < 0.001) is observed with one exception: there is no significant increase in apex loop diameters (P < 0.095). The observation in VO is the same as seen during H, namely, a significant increase in nail fold capillary diameters (P < 0.05) in both conditions. These data are displayed in Table 3.

Discussion

The main findings of this study are: 1) acute systemic hypoxia does not change capillary recruitment in nail fold capillaries, 2) nail fold capillaries increase in diameter in healthy young subjects, and 3) red blood cell velocity is not changed by hypoxic signal.

During systemic hypoxia, which causes arterial blood hypoxemia, an increase in capillary diameter in the nail fold area is observed (Table 3) without a change in red blood cell velocity (Table 2) in the basal hypoxic state and in the functional tests (reactive hyperemia and venous congestion). Our data demonstrating lack of changes in capillary recruitment is consistent with previous reports by Antonios et al. (1999) and Tibirica et al. (2009) when comparing control subjects. However, there are large capillary circulation differences in various skin areas, which can therefore be responsible for different adaptation mechanisms. For example, only 55% of the perfused capillaries in foot skin are used under normal conditions (Lamah et al., 2001), meaning that

Table 2
Nail fold capillaroscopy data of study subjects.

Characteristics	Normoxia (pO_2 = 21%) (n = 19)	Hypoxia (pO_2 = 12%) (n = 19)	P value (N vs. H)
MCD, capillaries/mm^2			
Baseline	22.6 ± 9.3	21.6 ± 8,2	0.13
Post-occlusive reactive hyperemia	24.2 ± 9.5*	22.5 ± 8.3	0.08
Venous occlusion	23.8 ± 9.1	22.6 ± 8.8	0.12
Absolute increase in MCD, capillaries/mm^2			
Post-occlusive reactive hyperemia	2 (−2; 7)	1 (−2; 6)	0.36
Venous occlusion	1 (−4; 7)	1 (−4; 7)	0.54
Relative increase in MCD, %			
Post-occlusive reactive hyperemia	8 (−8; 26)	5 (−8; 27)	0.25
Venous occlusion	6 (−9; 25)	4 (−14; 23)	0.27
	Normoxia (pO_2 = 21%) (n = 88)	Hypoxia (pO_2 = 12%) (n = 87)	
RBC velocity, mm/s			
Baseline	0.61 ± 0.26	0.63 ± 0.24	0.63

MCD, mean capillary density; RBC velocity, red blood cell velocity; N, normoxia; and H, hypoxia. Values represent means ± SD and median (minimum; maximum). *P < 0.05 vs baseline used paired Student's/Wilcoxon ranked-sum test.

recruitment is possible in this skin area, where it is needed. It is known that acute systemic hypoxia causes vasodilatation in the forearm (Leuenberger et al., 1999; Markwald et al., 2011) as well as cutaneous vasodilation (Simmons et al., 2007), and our data suggest vasodilation in the nail fold as well. The nail vascular bed consists of a very small area of skin, but our data indicate not only that it is responsible for acute adaptations, as in our case, but also that it is one of the skin areas which changes in the absence of systemic and peripheral diseases like hypertension (Serne et al., 2001), diabetes mellitus (Tibirica et al., 2009), Raynaud's phenomenon (Herrick, 2005) etc.

Our findings suggest that acute adaptation to a hypoxic signal is more related to capillary diameter change rather than functional density (functional tests) or capillary recruitment in the nail fold area. There is evidence that capillary recruitment, originally defined as an opening of previously closed capillaries in response to greater metabolic demands of tissue (Lamah et al., 2001), takes place in other vascular beds like muscles (Zhang et al., 2004) and even in other regions of the skin (Serne et al., 2002; Lamah et al., 2001). The acute adaptation in nail fold capillaries by increasing diameter could be typical just for this vascular bed, because in other beds like nonworking muscles, hypoxia causes a decrease in capillary diameters and reduces capillary blood flow (Bertuglia et al., 1991). However, in a recent study, Parthasarathi and Lipowsky (1999) reported that RBC deformability may adversely affect capillary recruitment and the physiological mechanisms that ensure adequate oxygen delivery to tissues. The direct mechanism of this change is unclear, with indications that determinants of capillary density could be active and/or passive mechanisms (Johnson, 1995). One active mechanism is via pericyte-mediated regulation of capillary diameter (Hamilton et al., 2010) established in the brain's vascular bed. Another possible mechanism is passive regulation of capillaries (Johnson, 1995) via flow motion (Rucker et al., 2000). The main mechanism regulating the number of functioning skin capillaries could be the activity of pre-capillary sphincters (Krupatkin, 2007) or arteriole vasomotion, which causes capillary flow motion (Rucker et al., 2000). Some data in animal models indicate that critical perfusion conditions in rat peripheral tissues induce arteriole vasomotion and capillary flow motion in muscle, but not in the periosteum, subcutis, and skin (Rucker et al., 2000). Furthermore, changes in recruitment in our study were not observed, which could be due to the small viewing area and low oxygen demand in resting normothermic conditions of the skin (Stücker et al., 2002), estimated to be 0.8 ml/min per 100 ml of skin tissue (Lamah et al., 2001). However, the transcutaneous gas (Table 1) measurements, especially carbon dioxide concentration (Kvarstein et al., 2003), indicate that metabolic demands of the skin were the same as before, but oxygen concentration was significantly decreased in acute systemic hypoxia.

Our results show no change in RBC velocity during decreased oxygenation of arterial blood, despite the fact that we observed an increase in capillary diameters. It is known that shape and diameter of capillaries greatly influence RBC velocity. Specifically, velocity has an inversely proportional relationship to capillary diameter, thus the velocity of RBCs is slower in wider than in narrower capillaries (Jeong et al., 2006). It is interesting that, in normoxic conditions, the mean RBC flow rate over a range of capillary diameters appears to be almost

Table 3
Nail fold capillary loop diameters.

Characteristics	Normoxia (pO_2 = 21%) (n = 52)	Hypoxia (pO_2 = 12%) (n = 52)	P value (N vs. H)
Apex, μm			
Baseline	15.5 ± 4.3	16.8 ± 4.3	0.002
Post-occlusive reactive hyperemia	17.9 ± 4.6*	18.4 ± 4.0*	0.095
Venous occlusion	17.3 ± 3.8*	18.2 ± 3.9*	0.048
Arterial limb, μm			
Baseline	11.9 ± 3.5	13.9 ± 4.1	0.000
Post-occlusive reactive hyperemia	13.9 ± 4.9*	16.8 ± 4.5*	0.000
Venous occlusion	14.4 ± 5.0*	16.8 ± 4.6*	0.000
Venous limb, μm			
Baseline	15.5 ± 4.3	17.2 ± 4.8	0.000
Post-occlusive reactive hyperemia	18.1 ± 4.8*	20.5 ± 5.4*	0.000
Venous occlusion	18.5 ± 5.5*	20.2 ± 4.8*	0.007
Total width, μm			
Baseline	39.9 ± 9.1	42.7 ± 10.3	0.000
Post-occlusive reactive hyperemia	42.4 ± 10.3*	46.0 ± 11.0*	0.000
Venous occlusion	41.8 ± 10.9*	44.4 ± 12.2*	0.006

Mean ± SD; N, normoxia; H, hypoxia; and *P < 0.05 vs baseline used paired Student's/Wilcoxon ranked-sum test. Capillary loop measurements after Allen et al., 2003.

constant. This indicates that even though RBCs flow though non-uniform and narrow capillaries, the flow rate remains almost constant (Jeong et al., 2006). If we follow this relationship, it results in increased RBC flow rate by acute systemic hypoxia, because our findings indicate that hypoxemia, in comparison to normoxia, induces capillary diameter increase while RBC velocity remains unchanged. Our assumption is not inconsistent with previously reported increases in peripheral blood flow during systemic hypoxia (MacLean et al., 1998), but there is controversial evidence that acute hypoxia reduces blood flow to the skin (Boutin et al., 2008). Some authors have found that hypoxia causes a significant decrease of RBC velocity in larger arterioles while velocity remains unchanged in small ones (Bertuglia and Giusti, 2005), consistent with our observations.

The systemic arterial pO_2 data indicated that acute systemic hypoxia induces arterial hypoxemia, which has been noted previously (Salman et al., 2005), and it has been suggested that arterial pO_2 distribution during systemic hypoxia is homogenous (Bertuglia and Giusti, 2005) and O_2 delivery is shifted from arterioles to capillaries (Bertuglia and Giusti, 2005). Bertuglia and Giusti (2005) proposed that this shift is mediated by a nitric oxide (NO) pathway, which plays an important role in hypoxia-induced increases of capillary perfusion by modulating lipid peroxide formation in endothelial cells.

Our results may be clinically relevant in widely recognized but not well-understood circulatory disturbances such as Raynaud's phenomenon. In our case, the patterns in capillary loops were normal, as there were no large or hemorrhagic loops observed in any of the subjects. Acute systemic hypoxia does not change the count of capillary loops, but only increases their diameters. While primary Raynaud's syndrome is idiopathic and complete mechanisms are not fully understood (Herrick, 2005), its pathogenesis is characterized by vasospasms leading to vasoconstriction (Cooke and Marshall, 2005), thus decreasing blood supply to the respective regions, resulting in tissue hypoxia. Two possible mechanisms can contribute to Raynaud's syndrome (Herrick, 2005). One of the mechanisms is a decrease in vasodilator capacity of local mechanisms, and the other is an increase of vasoconstrictor actions, possibly due to hyperactivation of the sympathetic nervous system causing extreme vasoconstriction of peripheral blood vessels (Herrick, 2005). Local endothelial cells normally release vasodilator agents or autacoids such as prostacyclin and nitric oxide (NO) as a response to changes in blood supply or hypoxia (Pohl, 1990). Our experiment demonstrates that systemic hypoxia causes local vasodilation in nail fold capillaries, indicating that when endothelial cells of small arterioles are functional, there is a normal response of autacoids affecting vascular tone and platelet function (Nathan and Singer, 1999).

In conclusion, two possible mechanisms for delivering optimal oxygen concentration to tissue in acute systemic hypoxia can be identified: shifting of blood flow from small arterioles to greater capillary use of oxygen delivery (Bertuglia and Giusti, 2005), and our observations of capillary diameter changes, which increase capillary blood flow.

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